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Developmental changes in organ systems of transforming anuran larvae : a thesis ...

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DEVELOPMENTAL CHANGES IN ORGAN SYSTEMS OF TRANSFORMING ANURAN LARVAE

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by

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INTRODUCTION

Metamorphosis is considered to be a sequence of post-embryonic developmental changes in non-repreductive structures of an organism by which a larva adapted to one mode of life is transformed into an adult adapted to a different mode of life (Frieden, 1961; Etkin, 1964; Cohen, 1966). Metamorphosis includes morphological changes, cellular differentiation, biochemical alterations, and molecular shifts.

Morphological changes are observed in nearly every organ system. The most noticeable morphological changes during anuran metamorphosis include loss of gills (lungs when present probably do not have respiratory function), loss of the horny beak and denticles, enlargement of the mouth and development of teeth and tongue, shortening of the intestine, loss of tail and development of limbs and a thick, glandular skin.

Cellular changes during metamorphosis are known to occur in muscles, cartilaginous bone, eyelids, lungs, tongue, tympanic membrane, opercular perforation, skin and pigments, liver, pancreas, intestine, and tail (Frieden, 1961).

Metamorphosis is accompanied by a general increase in enzyme activity. With few exceptions the developing tissues prepare for the demands of the approaching change in life habit by alterations of the chemical processes and reactions. New proteins are produced and there is an increase in biosyntheses. Frieden (1961) states that the extensive chemical alterations of metamorphosis either have direct adaptive value for the animal or serve as a basis for further morphological or chemical changes which have adaptive value. According to Witschi (1956), when the biochemical alterations are fully understood, they will prove directly adaptive to land living.

Despite an enormous amount of information on the biochemical and physiological changes of amphibian metamorphosis, information about the general anatomy of anuran larvae is not readily available. The present study is a comparison of the general morphological changes during metamorphic climax of three anuran species belonging to three different families. This anatomical information is designed to assist those wishing to correlate cellular and chemical events with the corresponding morphological events.

MATERIALS AND METHODS

The species used in this study were *Rana catesbeiana*, the bullfrog, *Hyla regilla*, the pacific treefrog, and *Bufo boreas*, the western toad. *Rana catesbeiana* tadpoles were collected in February, 1970, in a farm pond five miles north of Ione, California. *Hyla regilla* eggs and tadpoles were collected during March, 1970, inside the northern Stockton city limits from the swimming pool cover of a city home owner. *Bufo boreas* tadpoles were collected in April, 1970, at the edge of the water on the northwest side of Hogan Dam Reservoir.

All tedpoles were kept in plastic pans (9" X 13" X 3½") containing approximately 4 liters of water. The water was changed every 2 - 3 days. Boiled lettuce was fed *ad libitum*. Tadpoles reaching stage XX (Table I) were removed from the pans and placed in separate containers with little water. At appropriate stages of metamorphic climax, individuals were terminally anesthetized in 3% urethane for immediate dissection, or preserved in 10% formalin for later examination. At least 5 animals, either fresh or preserved, were dissected from each stage of each of the three species.

Bufo larvae in premetamorphic stages cannot be staged by the same characteristics as *Rana* or *Hyla* larvae because the legs in *Bufo* grow continuously while the legs in *Rana* and *Hyla* grow after the body has reached certain growth stages. However, during metamorphic climax, *Bufo* displays most of the morphological characteristics found in *Rana* and *Hyla*. In the present study the tadpoles were staged using the external morphological characteristics of metamorphic climax suggested by Taylor and Kollros (1946) for *Rana pipiens*. The specific morphological characteristics chosen for staging the three species are shown in Table I.

Metamorphic climax is usually considered to begin at stage XX (Etkin, 1964), but this study begins with stage XVIII to show the position of organs before the onset of metamorphosis.

In all cases drawings are intended as diagrammatic representations of the typical condition at each stage. Tadpoles selected for dissection were considered in the typical condition at the time that they displayed the characteristic used to denote the stage (Table I).

TABLE I

LATE METAMORPHIC STAGES FOR ANURAN LARVAE

STAGE	MORPHOLOGICAL CHARACTERISTICS
XVIII	The cloacal tail piece has disappeared.
XIX	The skin window is clear. The enclosed forelegs press outward causing added bulge to each side.
XX	One or both forelegs emerge.
XXI	The angle of the mouth has reached a point midway between the nostril and the anterior margin of the eye.
XXII	The angle of the mouth has reached the level of the middle of the eye.
XXIII	The angle of the mouth has reached the level of the posterior margin of the eye.
XXIV	The mouth gape is complete. A dark tail stub remains.
XXV	The tail is completely gone. No dark tissue remains.

(Adapted from Taylor and Kollros, 1946)

OBSERVATIONS

Digestive Tract

The intestine is the most prominent organ in the larval body. In the three species studied, the intestine is arranged in a double spiral, winding dorso-ventrally in several loops as the spiral becomes narrower. In the center of this double spiral a "mesenteric stalk" (consisting of arteries, veins, and mesenteries) extends dorso-ventrally supplying blood and support to all parts of the intestine (Figures 2, 11).

At stage XVIII, the larval foregut is a simple tube which later will differentiate into the longitudinally infolded esophagus, muscular stomach and pyloris.

The size of the intestinal coil causes the other organs to be pushed to the sides of the body cavity. The liver lobes are pressed together on the extreme right side of the body cavity. The right lobe is partly hidden from ventral surface view as it lies dorsally above the gall bladder and the left liver lobes (Figures 1, 3, 5). The foregut leads posteriad from the pharynx on the right side of the body cavity and curves around the large pancreas forming the manicotto (larval anuran stomach) which leads into the midgut or small intestine (Figures 2, 3, 6). At stage XVIII the esophagus of each species shows some folds indicating widening and differentiation of the passage.

In R. catesbeina and H. regilla the common bile duct originates in the liver from the gall bladder and hepatic ducts and extends from the gall bladder on the extreme right abdominal wall into the intestinal mesenteries in the left abdominal cavity where it connects to the small intestine (Figures 2, 4). In B. boreas the common bile duct leads into the small intestine immediately posterior to the maincotto (Figure 6). Early and late stages XX in H. regilla show the common bile duct greatly reduced as compared to stage XVIII (Figures 11, 12).

Regression of the intestine is evident in H. regilla at stage XIX (Figure 8). As the intestine shortens, the liver lobes move to the middle of the body cavity (Figure 24). Some reduction in the intestine is noticeable in B. boreas at stage XIX, while both B. boreas and R. catesbeiana show considerable shortening of the intestine in stage XX Figures 9, 13). Intestinal regression is apparently complete in H. regilla by late stage XX.

In R. catesbeiana the common bile duct, the small intestine and the accompanying mesenteries regress, causing the bile duct to move in circular rotation about its point of origin in the liver (Figures 2, 10, 15, 16). By stage XXII the duct is close to the length and position which it reaches by stage XXV. As the intestine takes less space, the liver lobes move toward the center of the body cavity. This regression of the digestive tract and the rotation of the liver give the transforming R. catesbeiana and

H. regilla a narrowing "waistline", especially noticeable in H. regilla. Intestinal regression in B. boreas occurs at approximately the same stages as that of R. catesbeiana.

In B. boreas, stages XXII and XXIII, the remnants of the larval double spiral can be seen in the twist of the intestine (Figures 17, 20). R. catesbeiana also retains remnants of the larval double spiral in the intestine at stage XXV (Figure 21).

At stage XVIII the gall bladder is located on the right side of the body cavity in association with the liver. As the liver moves to the center of the abdominal cavity, the gall bladder moves with it (Figures 9, 14, 18, 21). By stage XXV the gall bladder is filled with a dark fluid in all three species.

As intestinal regression occurs, the position of the manicotto changes. As it shortens and becomes more muscular, it rotates along with the liver and the rest of the digestive tract into the adult position (Figures 10, 15, 16, 18).

Pancreas

At stage XVIII the larval pancreas is large in relation to other body parts (Figures 2, 3, 6). The anterior portion is located between the right and left liver lobes lateral to the gall bladder. The bulk of the organ extends along the curve of the manicotto and a short portion of the small intestine (Figures 2, 6). During metamorphic climax, the pancreas degenerates to very little tissue which may be difficult to find in stages XXII and XXIII and then is reformed into a relatively smaller organ (Table II). In *H. regilla* at stage XXII and *B. boreas* at stage XXIII, a few individuals apparently lacked a formed pancreas.

Gills and Lungs

The gills contain highly branched tufts of tissue which contain many small blood vessels. Each gill is surrounded by a tough, clear membrane, the gill sac, inside of which the forelegs form. The right gill area is connected to the left by a narrow opening directly ventral to the heavily pigmented pericardium. In the three species considered, water taken in by the mouth passes through the pharynx, through the slits in the branchial cartilages and over the gill arches, then out the spiracle on the left side (Figure 7).

The stage at which the lungs are used varies among species. *Rana catesbeiana* is known to gulp air in the premetamorphic stages. The lungs extend the full length of the body cavity and are partly inflated well before metamorphic climax. At late stage XXII *R. catesbeiana* no longer has blood circulation through the gills (Table III) as the lungs become inflated, shorter, and wider with prominent blood vessels (Figure 18, 21).

At stage XVIII *B. boreas* possesses only small buds of lungs which increase rapidly in size. By stage XXIII the *Bufo* gills are cut off from the blood supply and the

TABLE II

APPEARANCE OF THE PANCREAS DURING METAMORPHIC CLIMAX

STAGE	*R. catesbeiana*	*H. regilla*	*B. boreas*
XVIII	++++	++++	++++
XIX	++++	+++	++++
XX	+++	++	++++
XXI	++	++	+++
XXII	++	+ (0)	++
XXIII	+	+++	+ (0)
XXIV	++	++++	++
XXV	++++	++++	++++

Symbols:

++++	Large larval pancreas or normal adult organ
+++	Reduced larval pancreas or increased reformed pancreas
++	Small organ
+	Some tissue present
(0)	Pancreatic tissue apparently absent in gross examination

lungs begin functioning (Table III). Development continues until the lungs are highly inflated, wide, vascular structures by the end of metamorphosis.

Hyla regilla enters metamorphic climax with long partially inflated lungs (almost the length of the body cavity). Regression of the gills is somewhat faster in H. regilla than in the other two species. The lungs become inflated, wider, and more vascular by stage XXII when the gills cease all function (Table III). The lungs are highly inflated in the later stages (Figures 19, 22).

Bladder

The urinary bladder increases in size during the later stages of metamorphosis. The bladder is an evagination on the ventral wall of the cloaca adjacent to the openings of the Wolffian ducts. It is a highly folded membrane with much expension potential, especially in B. boreas. In R. catesbeiana a bocornuate bladder develops by the late stages of metamorphosis. The H. regilla bladder consists of one lobe. The B. boreas bladder is bilobed, each lobe attached by mesenteries to the dorsal peritoneum (Figures 20, 23).

Fat Bodies

R. catesbeiana and H. regilla tadpoles have massive fat bodies in the abdominal cavity. Some reduction of the massive fat bodies by stage XXIV and XXV was noticed. Though most of the B.boreas larvae dissected had no fat bodies evident, six had one or two very small lobes of

TABLE III

APPEARANCE OF GILLS AND LUNGS DURING METAMORPHIC CLIMAX

GILLS	*R. catesbeiana*	*H. regilla*	*B. boreas*
Apparently functional	XVIII XIX	XVIII XIX	XVIII XIX
Regression apparent	XX	XX	XX XXI
Marked regression	XXI	XXI	XXII
No blood supply	Late XXII	XXII	XXIII
Tissue remnants	XXIII	XXIII	XXIV
LUNGS			
Long,* pigmented, partial inflation	XVIII XIX XX	XVIII XIX	
Short,* clear, partial inflation			XVIII XIX XX
Long,* inflated, vascular increase	XXI	XX XXI	
Short,* clear, inflated, vascular increase			XXI XXII
Medium length,* wide, inflated	XXII XXIII XXIV XXV		XXIII XXIV XXV
Long,* wide, inflated		XXII XXIII XXIV XXV	

*Note: Long lungs extend 3/4 or more the length of the body cavity.
Medium length lungs extend 1/2 - 3/4 the length of the body cavity.
Short lungs extend less than 1/2 the length of the body cavity.

fat attached to the undifferentiated gonads on the anterior kidney. Two of these were stage XVIII; two were stage XIX; one was stage XXIV; and one was stage XXV.

Orbitohyoid Muscle

The orbitohyoid muscle is prominent just under the skin anterior to the heart. This strong larval muscle attaches to the processes of the hyoid arch and is visible in all the earlier metamorphic stages in all three species (Figures 1, 3, 5). By stage XXIII in each species, the orbitohyoid muscle is either very thin or has completely disappeared (Figures 18, 19, 20).

As the tadpole transforms, the small mouth in stage XX begins to develop into the large adult mouth. The mandibular cartilages of the lower jaw grow in length posteriad until by stage XXIII they reach the hyoid processes to which the orbitohyoid muscle attaches (Figures 17, 20, 23). By stage XXIV they have grown ventrally over the hyoid cartilage, which can no longer be seen from the ventral surface.

Thyroid Glands

The thyroid gland was easily found in all stages of *R. catesbeiana* and *H. regilla* except for stage XXV. In the later stages, when the laryngo-tracheal cartilage and the pharyngeal cartilage are developing, the thyroids are carried with the developing cartilage into slightly altered orientation. The glands are farther apart at stage XXV than at stage XVIII, with additional tissue ventral to them (Figures 1, 21). The glands are found slightly anterior to the larynx and between the larynx and the jugular vein in a recess between processes of the pharyngeal cartilage.

Bufo boreas thyroid glands are difficult to find in all stages because the throat area is deep and contains pigmented membrances. The thyroid location is shown in two diagrams (Figures 5, 23), although the glands cannot be seen from the ventral surface without probing. During the later stages of metamorphosis the gland occupies a recess in the pharyngeal cartilage. The tissues ventral to the glands contain black pigment.

DISCUSSION

The three anuran species in this study follow the same metamorphic events, though with some difference in the relative rate of progress through the stages. Tadpole stages are based upon external morphological characteristics, not necessarily correlated with internal events. *H. regilla* progresses faster than do the other two species in regression and development of the digestive tract, in degeneration and reforming of the pancreas, and in the regression of the gills and development of the lungs. These systems develop earlier than modification of the mouth and regression of the tail on which the stages are based.

The larval foregut is essentially a long uniform tube in which no digestion takes place. There is no peristaltic movement in the larval foregut. Food is moved from the pharynx to the manicotto (a storage organ) by ciliary action (Reeder, 1964). The long, narrow intestinal tube increases the absorbing surface of the tract, necessary for the digestion of the predominently herbivorous diet (Noble, 1931).

Regression and differentiation of the digestive tract appear concurrently throughout all parts at the same time. The esophagus becomes shorter as it develops folds; the manicotto differentiates into the broad muscular stomach; the small intestine shortens and develops many longitudinal and transverse folds which increase the absorptive surface (Noble, 1931); the large intestine becomes very short with an anterior enlarged reservoir for feces at the juncture of the small intestine. It has been suggested that the slight assymmetrical enlargement of the anterior reservoir represents a rudimentary coecum (Noble, 1931).

In Rana and Hyla the long common bile duct of the larvae regresses with the intestine until the juncture of the duct and the intestine reach a point just posterior to the stomach under the liver. In Bufo the juncture of the short bile duct to the intestine occurs at the end of the foregut. Intestinal regression apparently takes place posterior to the juncture of the bile duct with the intestine.

Early studies on Bufo show that regression of the intestine apparently progresses from both ends through the gradual contraction and reorganization of the circular and longitudinal muscle (Reeder, 1964).

The complete degeneration and reforming of the pancreas has been reported from studies on Xenopus (Deuchar, 1966). The present study indicates that the transforming tadpole may have little or no pancreatic tissue for a short time during one stage. New tissue may be forming as the larval organ degenerates so that most individuals have pancreatic tissue at all times throughout metamorphosis. Digestive enzymes are only briefly interrupted during the non-feeding period of the transformation (Frieden, 1961; Reeder, 1964). Amphibian islet tieeur shows anatomical and functional differentiation late in metamorphosis (Noble, 1931). Insulin secretion appears at the end of metamorphosis when the liver begins to store glycogen (Deuchar, 1966).

The stage at which the lungs begin to function is difficult to pinpoint in a general morpholgical study such as this. It is probable that the lungs in Rana and Hyla assume some respiratory function before the gills regress to the non-functional condition. The lungs may serve as hydrostatic organs as well as respiratory organs (Noble, 1931; Witschi, 1949, 1956). Foxon (1964) reports that some Rana species gulp air very early and that Bufo species do not use their lungs until metamorphosis. The lungs in Bufo boreas are very short, narrow structures until the onset of metamorphosis when development occurs rapidly. After metamorphosis Bufo is a terrestrial creature with large highly inflated lungs.

The urinary bladder, especially in Bufo, expands to store large quantities of water. The dorsal position of the cloaca and the bladder in the lower body cavity of B. boreas allows space for great expansion to the lateral and ventral walls. The bilobed bladder may extend into the space on each side of the lower body cavity. The amphibian bladder has been shown to be significant in problems of water balance since water may be absorbed through the bladder walls if the animal becomes dehydrated (Deyrup, 1964).

SUMMARY

Hyla regilla proceeds through certain metamorphic events earlier than *Rana catesbeiana* and *Bufo boreas*. Those changes observed in this study were digestive tract regression and development, pancreas degeneration and reformation, gill regression and lung development.

The liver appears to unfold and expand as it rotates from the larval position against the right abdominal wall to the adult position in the center of the abdominal cavity.

The body contour of *Bufo boreas* tends to be influenced by the large inflated lungs in the upper body cavity and the space available for water storage in the bladder in the lower body cavity. The development of these structures appears to give the toad adaptive advantage for a more terrestrial adult life.

In *Rana catesbeiana* and *Hyla regilla* the common bile duct of premetamorphic larvae is long, extending completely across the abdominal cavity. During metamorphosis the bile duct shortens as intestinal regression and liver rotation bring the gall bladder and small intestine into close proximity. In *B. boreas* the junction of the common bile duct with the small intestine is close to the gall bladder even in premetamorphic stages, so the bile duct is short at all stages.

The larval pancreas degenerates and reforms into the smaller adult pancreas during metamorphic climax. Most individuals have at least some pancreatic tissue during this process, though a few appear to have none under gross examination.

LITERATURE CITED

Cohen, P.P. 1966. Harvey Lectures. 60: 119-154.

Deuchar, E.M. 1966. Biochemical Aspects of Amphibian Development. John Wiley and Sons Inc., New York.

Deyrup, Ingrith J. 1964 Water balance and kidney. In Physiology of the Amphibia. Ed. J.A. Moore. Academic Press, New York.

Etkin, W. 1964. Metamorphosis. In Physiology of the Amphibia. Ed. J.A. Moore. Academic Press, New York.

Foxon, G.E. 1964. Blood and respiration. In Physiology of the Amphibia. Ed. J.A. Moore. Academic Press, New York.

Frieden, E. 1961 Biochemical adaptation and anuran metamorphosis. Am. Zool. 1: 115-149.

Noble, G.K. 1931. The Biology of the Amphibia. Dover Ed. 1955. Dover Publications, Inc., New York.

Reeder, W.G. 1964. The digestive system. In Physiology of the Amphibia. Ed. J.A. Moore. Academic Press, New York.

Taylor, A.C. and Kollros, J.J. 1964. Stages in the normal development of <u>Rana pipiens</u> larvae. Anat. Rec. 94: 7-23.

Witschi, E. 1949. The larval ear of the frog and its transformation during metamorphosis. Zeig. f. Naturforschung 4b: 230-242.

Witschi, Emil. 1956. Development of Vertebrates. W.B. Saunders Co., Philadelphia.

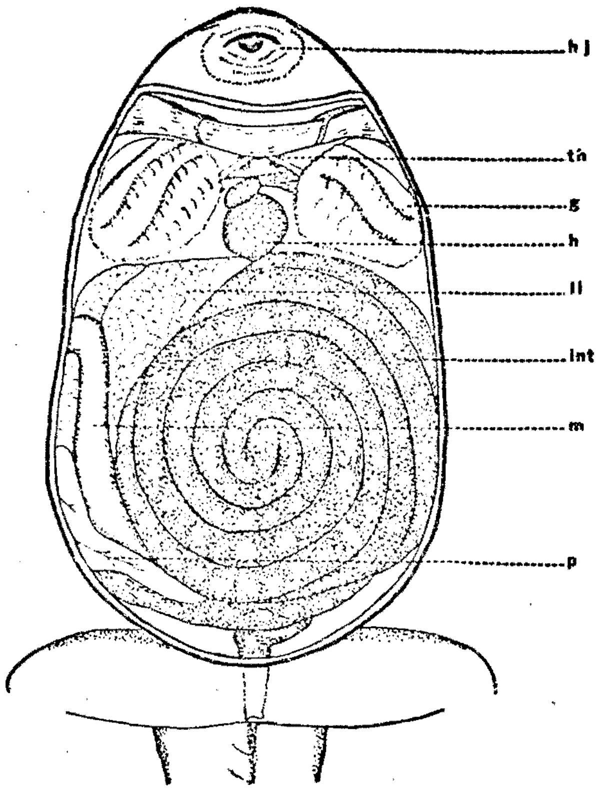

Figure 1. A Superficial View of the Ventral Internal Organs of R. catesbeiana, Stage XVIII. Symbols: h j, horny jaws; th, thyroid gland; g, gill; h, heart; li, liver; int, intestine; m, manicotto; p, pancreas.

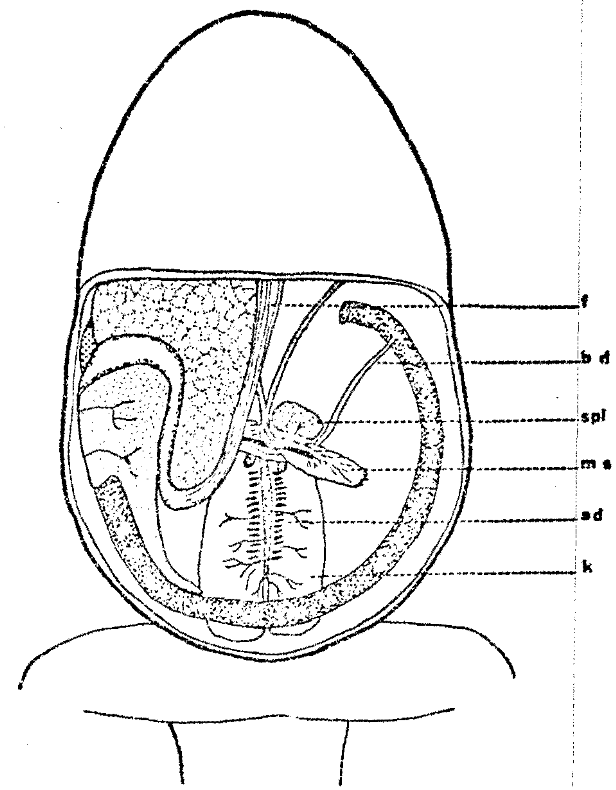

Figure 2. View of Internal Organs with Most of the Intestine Removed in R. catesbeiana, Stage XVIII. Symbols: f, foregut; b d, common bile duct; spl, spleen; m s, mesenteric stalk; ad, adrenal gland; k, kidney.

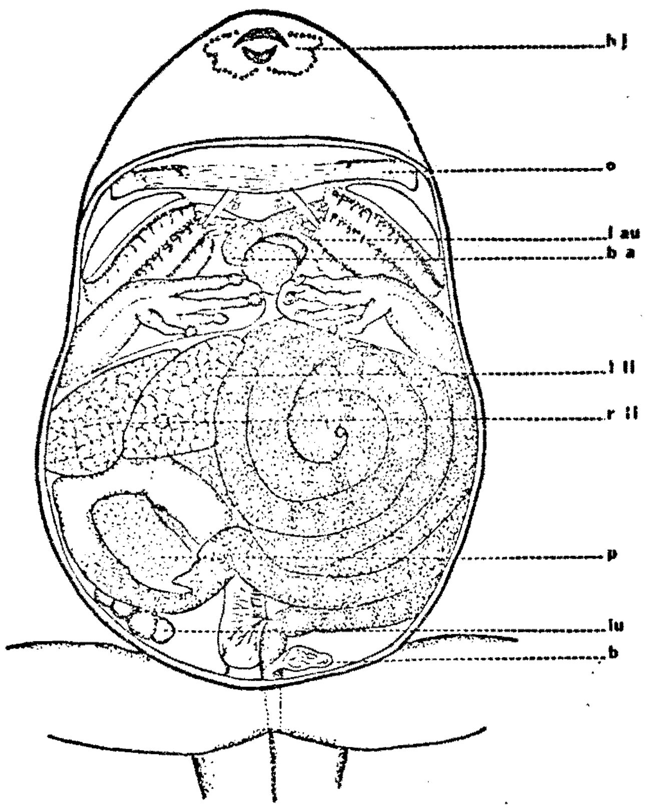

Figure 3. A Superficial View of the Ventral Internal Organs in H. regilla, Stage XVIII. Symbols: h j, horny jaws; o, orbitohyoid muscle; l au, left auricle; b a, bulbous arteriosus; l li, left liver lobes; r li, right liver lobe; p, pancreas; lu, lung; b, bladder.

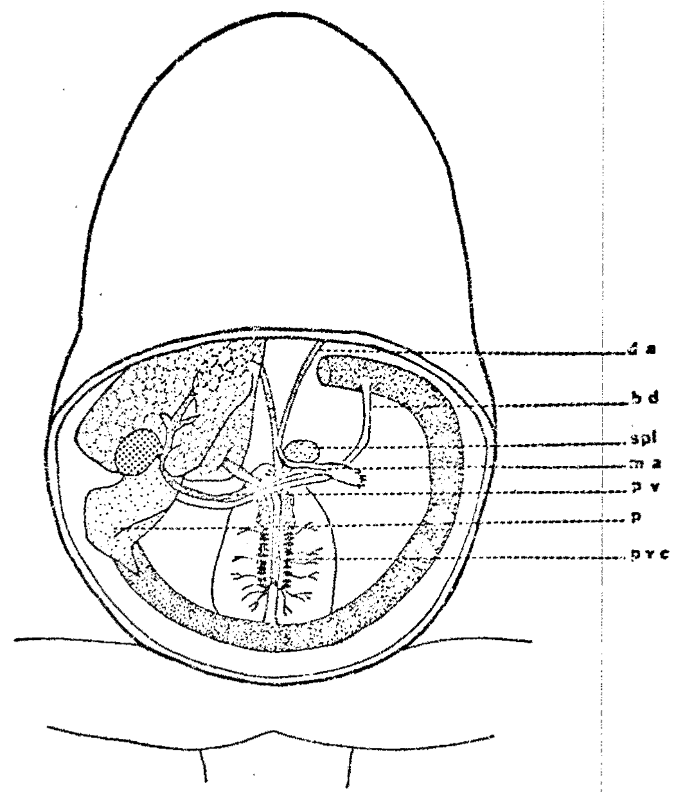

Figure 4. A View of the Internal Organs with Foregut and Most of the Intestine Removed in H. regilla, Stage XVIII. Symbols: d a, dorsal aorta; b d, bile duct; spl, spleen; m a, mesenteric artery; p v, portal vein; p, pancreas; p v c, posterior vena cava.

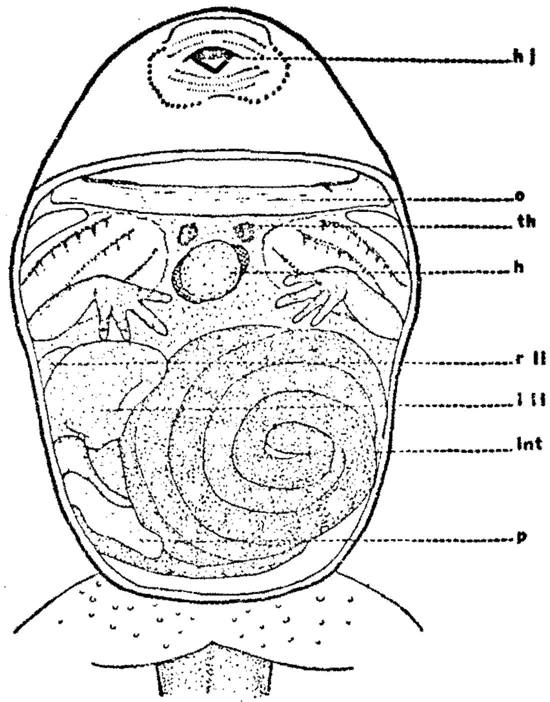

Figure 5. A Superficial View of the Ventral Internal Organs in B. boreas, Stage XVIII. Symbols: h j, horny jaws; o, orbitohyoid muscle; th, thyroid gland; h, heart; r li, right liver lobe; l li, left liver lobes; int, intestine; p, pancreas.

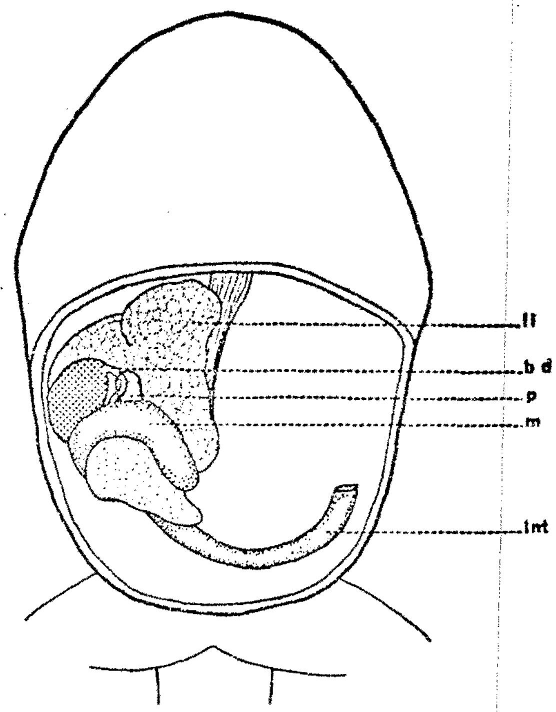

Figure 6. The Common Bile Duct in Relation to Other Internal Organs with Most of the Intestine Removed in B. boreas, Stage XVIII. Symbols: li, liver; b d, common bile duct; p, pancreas; m, manicotto; int, intestine.

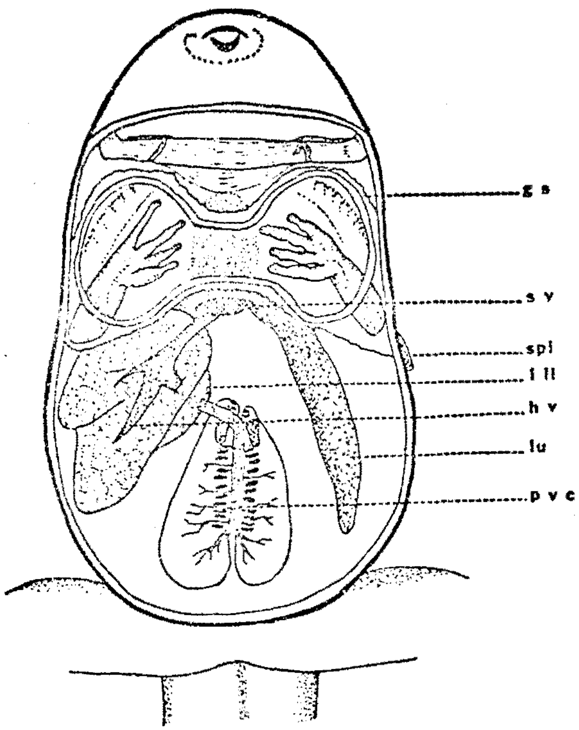

Figure 7. The Ventral Internal Organs and Gill Sacs with Connecting Canal and Spiracle in R. catesbeiana, Stage XIX. Symbols: g s, gill sac; s v, sinus venosus; spi, spiracle; l li, left liver lobes turned to show dorsal surface; h v, hepatic vein; lu, lung; p v c, posterior vena cava.

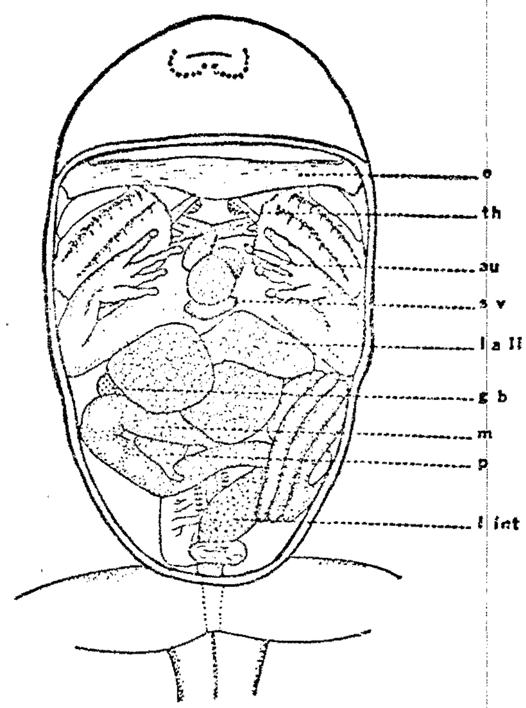

Figure 8. A Superficial View of the Internal Organs in H. regilla, Stage XIX. Symbols: o, orbitohyoid muscle; th, thyroid gland; au, auricle; s v, sinus venosus; l a li, left anterior liver lobe; g b, gall bladder; m, manicotto; p, pancreas; l int, large intestine.

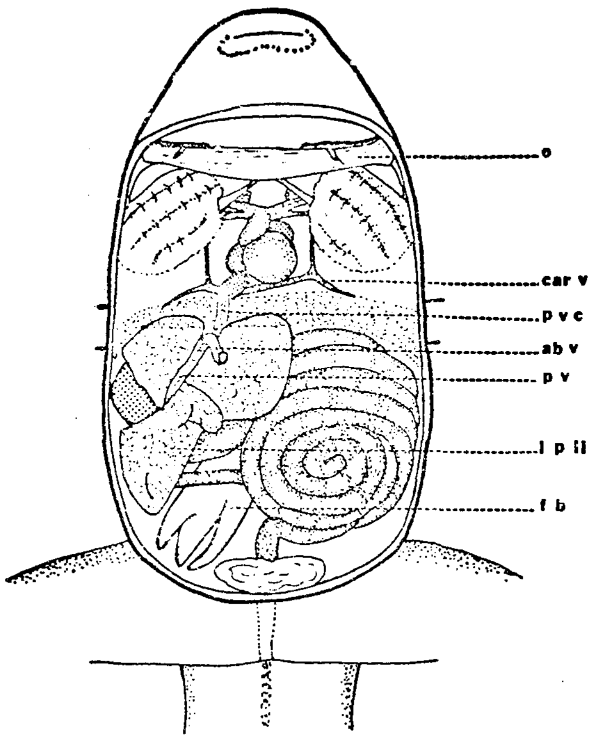

Figure 9. A Superficial View of the Internal Organs in R. catesbeiana, Stage XX. Symbols: o, orbitohyoid muscle; car, common cardinal vein; p v c, posterior vena cava; ab v, abdominal vein; p v, portal vein; l p li, left posterior liver lobe; f b, fat body.

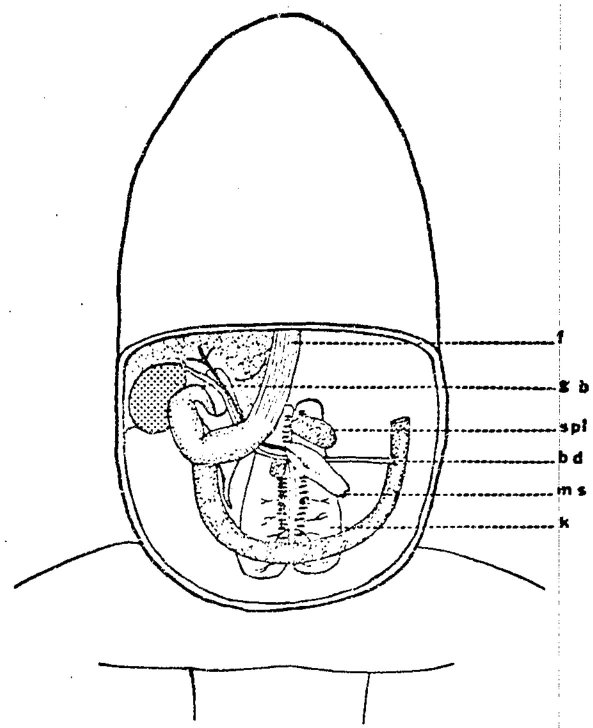

Figure 10. The Common Bile Duct in Relation to Other Internal Organs in R. catesbeiana, Stage XX. Symbols: f, foregut; g b, gall bladder; spl, spleen; b d, bile duct at juncture with intestine; m s, mesenteric stalk; k, kidney.

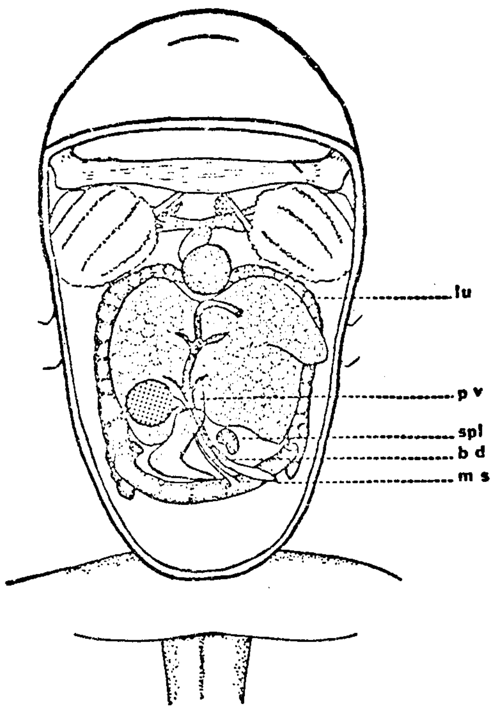

Figure 11. Liver Lobes Spread Apart to Show Length of Common Bile Duct in *H. regilla*, Early Stage XX. Symbols: lu, lung; p v, portal vein; spl, spleen; b d, bile duct; m s, mesenteric stalk.

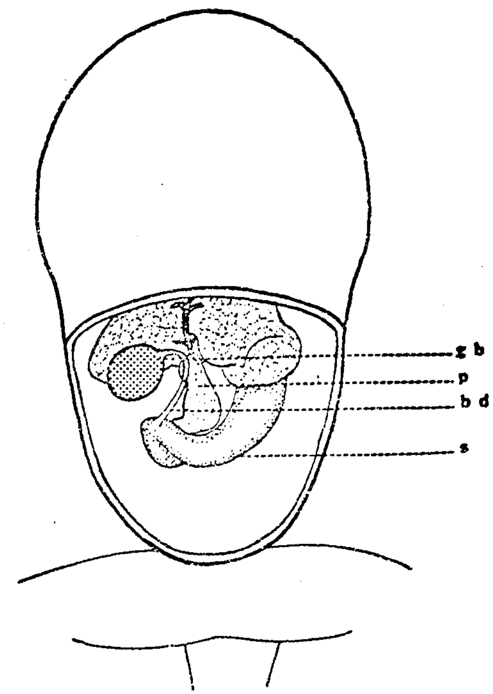

Figure 12. Liver Lobes Spread Apart to Show Regression of Common Bile Duct in *H. regilla*, Late Stage XX. Symbols: g b, gall bladder; p, pancreas; b d, bile duct; s, stomach.

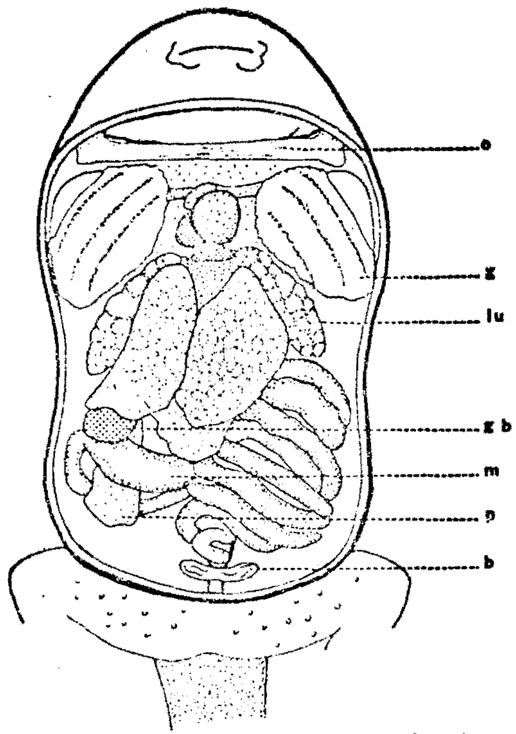

Figure 13. Regression of the Intestine in Relation to Other Organs in B. boreas, Stage XX. Symbols: o, orbitohyoid muscle; g, gill; lu, lung; g b, m, manicotto; p, pancreas; b, bladder.

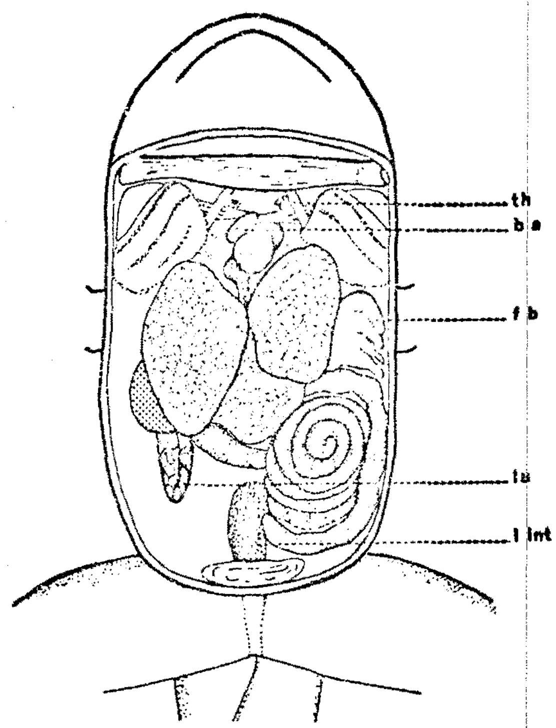

Figure 14. Regression of the Intestine in Relation to Other Organs in R. catesbeiana, Stage XXI. Symbols: th, thyroid; b a, bulbous arteriosus; f b, fat body; lu, lung; l int, large intestine.

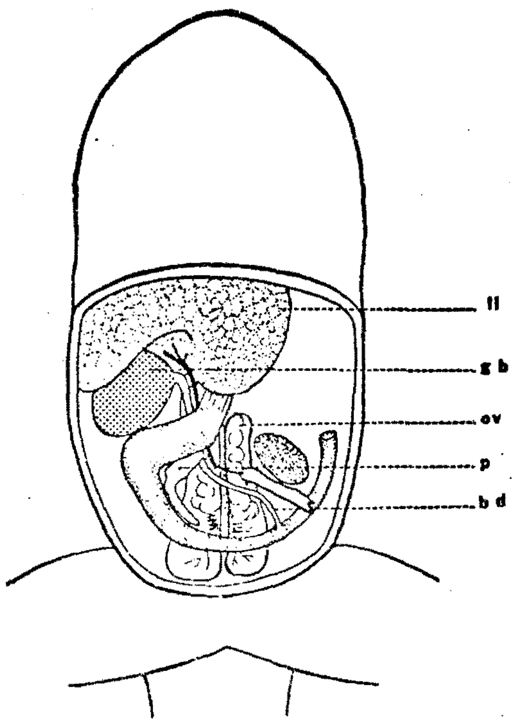

Figure 15. Liver Lifted to Show Position of Common Bile Duct in R. catesbeiana, Stage XXI. Symbols: li, liver; g b, gall bladder; ov, ovary; p, pancreas; b d, bile duct.

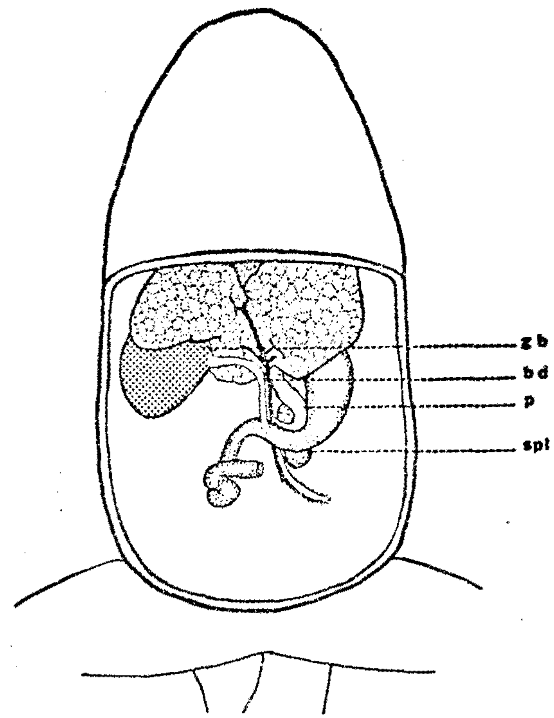

Figure 16. Liver Lifted to Show Position of Common Bile Duct in R. catesbeiana, Stage XXII. Symbols: g b, gall bladder; b d, bile duct; p, pancreas; spl, spleen.

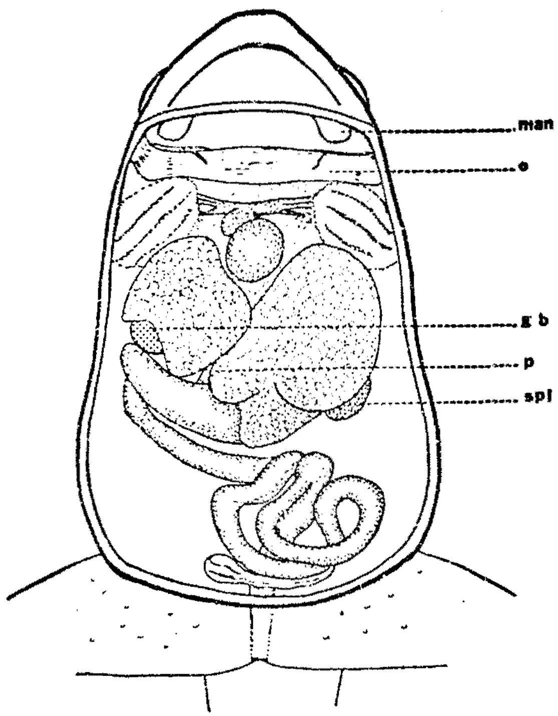

Figure 17. A Superficial View of the Ventral Internal Organs and the Growing Mandible Cartilages in B. boreas, Stage XXII. Symbols: man, mandible; o, orbitohyoid muscle; g b, gall bladder; p, pancreas; spl, spleen.

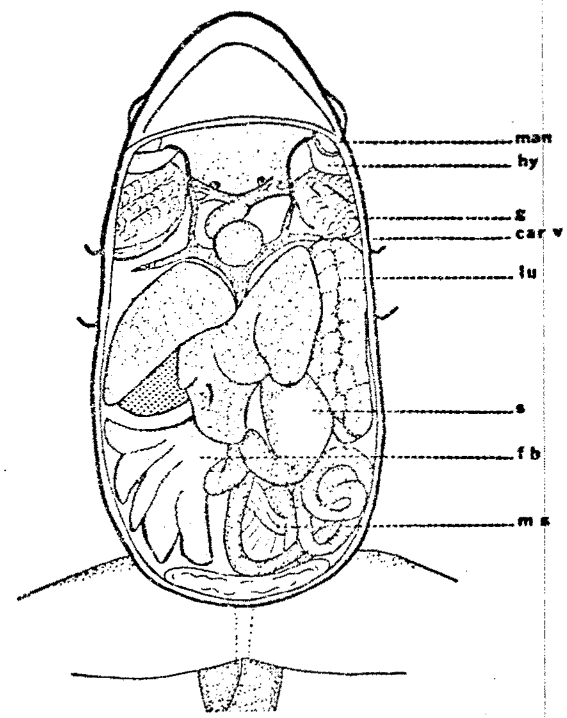

Figure 18. The Ventral Internal Organs and Gill Tissue Remnants in R. catesbeiana, Stage XXIII. Symbols: man, mandible cartilage: hy, hyoid cartilage; g, gill tissue remnants; car v, cardinal vein; lu, lung; s, stomach; f b, fat body; m s, mesenteric stalk.

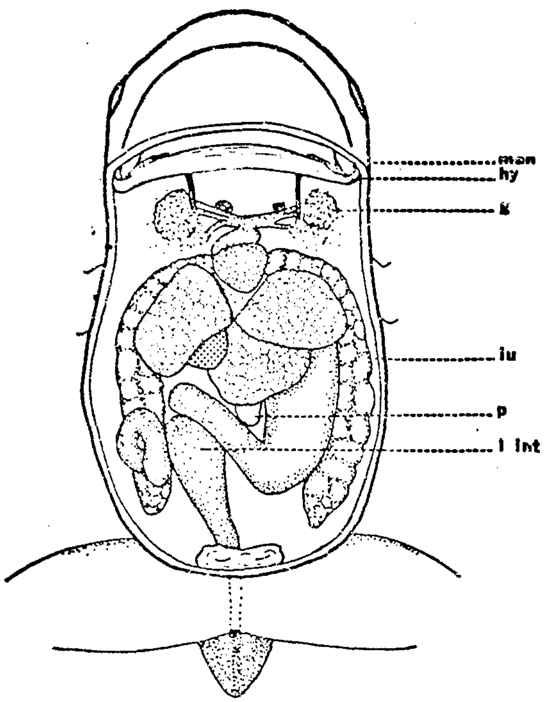

Figure 19. The Ventral Internal Organs and Gill Tissue Remnants in H. regilla, Stage XXIII. Symbols: man, mandible cartilage; hy, hyoid cartilage; g, gill tissue remnants; lu, lung; p, pancreas; l int, large intestine.

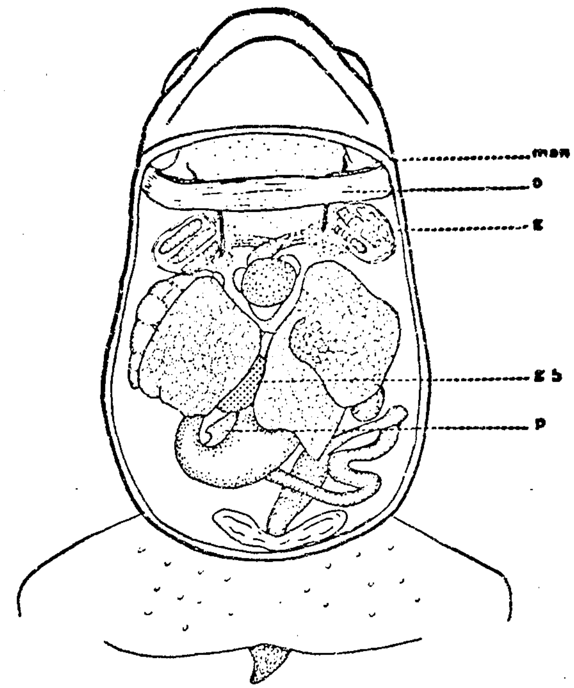

Figure 20. The Ventral Internal Organs and Gill Tissue Remnants in B. boreas, Stage XXIII. Symbols: man, mandible cartilage; o, orbitohyoid muscle; g, gill tissue remnants; g b, gall bladder; p, pancreas.

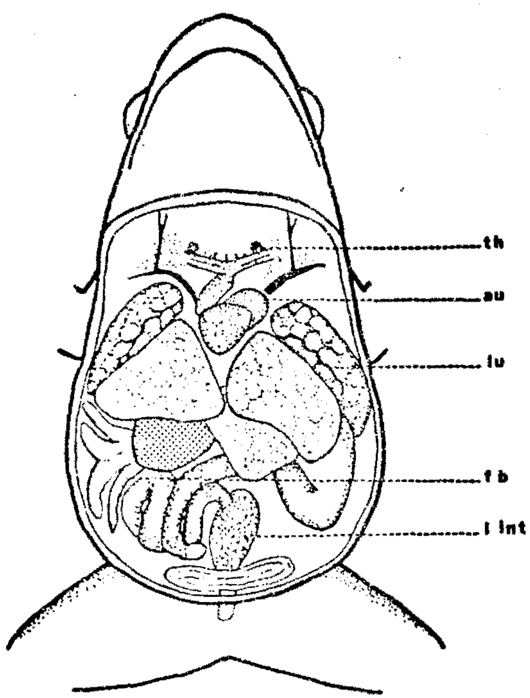

Figure 21. A Superficial View of the Ventral Internal Organs in R. catesbeiana, Stage XXV. Symbols: th, thyroid gland; au, auricle; lu, lung; f b, fat body; l int, large intestine.

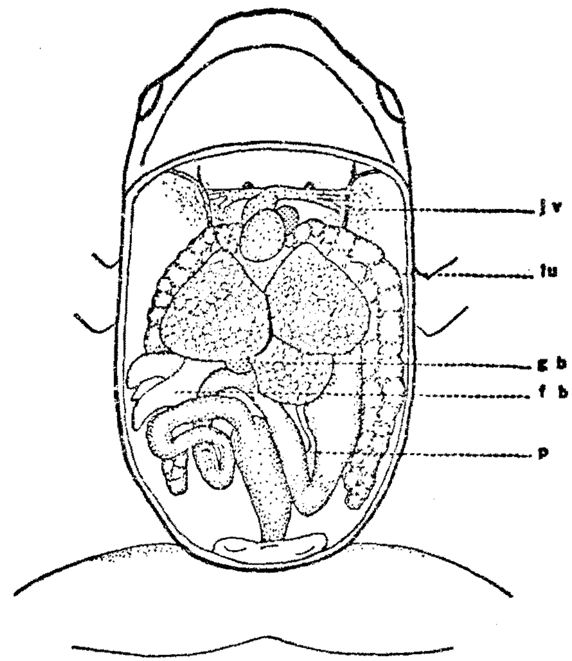

Figure 22. A Superficial View of the Ventral Internal Organs in H. regilla, Stage XXV. Symbols: j v, external jugular vein; lu, lung; g b, gall bladder; f b, fat bodies; p, pancreas.

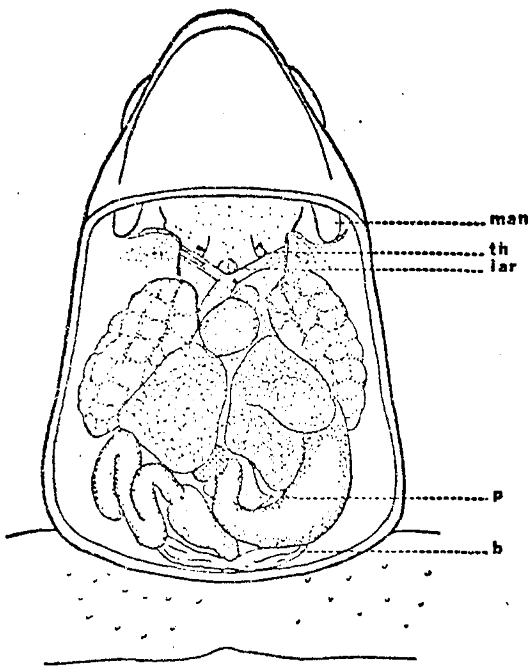

Figure 23. A Superficial View of the Ventral Internal Organs in B. boreas, Stage XXV. Symbols: man, mandible cartilage; th, thyroid; lar, larynx; p, pancreas; b, bladder.

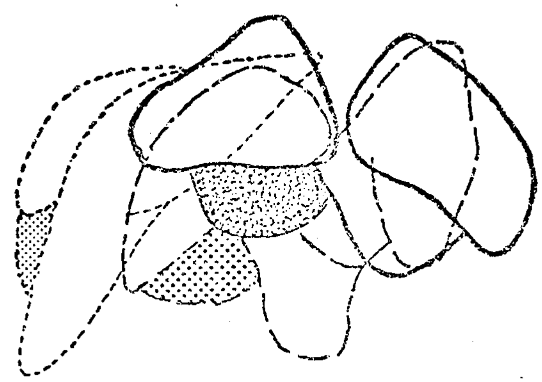

Figure 24. Rotation of Liver and Gall Bladder in R. catesbeiana, Stages XVIII, XXIII, XXV.
Stage XVIII --------
Stage XXIII — — —
Stage XXV ———
Shaded areas represent the gall bladder.